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# ***Diaminorhodamine-4M acetoxymethyl ester (DAR-4M AM)***

Table 1. Product information

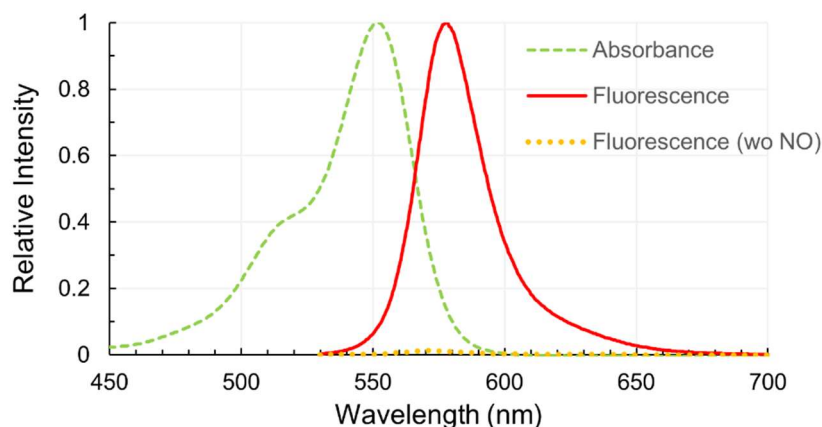
Catalog no.	Product name	Amount	Storage upon receipt	Stability
SK1006-01	Diaminorhodamine-4M acetoxymethyl ester (DAR-4M AM)	1 mg (in DMSO 0.32 mL)	≤-20°C, keep desiccated and protected from light.	1 year (when unopened and stored as described.)

## **1. About DAR-4M AM**

DAR-4M AM is an acetoxymethyl ester derivative of diaminorhodamine-4M. Cell permeable DAR-4M AM is hydrolyzed by intracellular esterase to generate cell impermeable DAR-4M. Thus it can detect intracellular NO in pH range of 4 – 12.

## **2. Properties of the reagent**

The reagent is provided as a solution in dimethyl sulfoxide (DMSO). It has almost no fluorescence but shows orange fluorescence upon reaction with NO, with maximum emission at 578 nm. Optimum excitation wavelength is 552 nm and it can be excited with either 532 nm or 543 nm laser. For observation with fluorescence microscopy, green excitation filter sets such as that for Cy3 are appropriate.



## **3. Preparation of the reagent**

The reagent is a solution of 5 mM. To avoid moisture absorption, warm the vial to the room temperature before opening the cap. Dilute the solution 500-fold with neutral buffer solution such as PBS (pH=7.4) to prepare working solution of final 10 μM. The concentration of the reagents should be optimized depending on the purpose of the experiment and the NO concentration to be detected.

#### 4. Precautions

- 1) Prepare working solutions just before use.
- 2) The quality of the reagent maybe compromised after opening the cap. Avoid repeated freeze-thaw cycles which may reduce the performance of the reagent.
- 3) Use neutral buffer (pH=7 to 7.5) to dilute the reagent. Addition of bovine serum albumin (BSA), phenol red, calcium ion and vitamins may affect the fluorescence.
- 4) The reagent is dissolved in dimethyl sulfoxide which is flammable. Handle with necessary precautions.

#### 5. References

1. Kojima, H., Hirotsani, M., Nakatsubo, N., Kikuchi, K., Urano, Y., Higuchi, T., Hirata, Y., Nagano, T. (2001)  
*Anal. Chem.*, **73**:1967 – 1973

Table 2. Related Products

Catalog no.	Product name	Major applications
SK1001-01	DAF-2	Detection of NO via green fluorescence.
SK1002-01	DAF-2 DA	Detection and imaging of intracellular NO via green fluorescence.
SK1003-01	DAF-FM	Detection of NO via green fluorescence (in pH $\geq 6$ )
SK1004-01	DAF-FM DA	Detection and imaging of intracellular NO in pH $\geq 6$ , via green fluorescence.
SK1005-01	DAR-4M	Detection of NO in pH range of 4-12 via orange fluorescence.